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EFFECT OF SELENIUM AND N-(P-AMYLCINNAMOYL) ANTHRANILIC ACID ON DOXORUBICIN-INDUCED KIDNEY INJURY IN RATS

Sıçanlarda Doksorubisin Kaynaklı Böbrek Hasarı Üzerine Selenyum ve N-(p-Amilsinnamoil) Antranilik Asit'in Etkisi

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ABSTRACT

Doxorubicin (DOXR) is one of the essential antitumor drugs. However, its serious adverse effects in many organs limit the clinical use of DOXR. This study aimed to investigate the effect of selenium (Se) and N-(p-Amilcinnamoyl) anthranilic acid (ACA) on kidney tissue in DOXR-administered rats. The rats in the study were divided into six groups (n=10); Control, DMSO, DOXR, DOXR+Se, DOXR+ACA and DOXR+Se+ACA. At the end of the study, intracardiac blood was drawn from rats, and kidney tissues were removed. Urea and creatine levels were measured in serum samples of rats. In addition, histopathological examination of kidney tissue was determined by H&E staining, and 8-OHdG expression was determined by immunohistochemical analysis. Urea and creatine levels increased with DOXR decreased in serum samples after Se and ACA treatments ($p< 0.05$). While glomerular atrophy, tubular and glomerular dilatation, vascular occlusion and degeneration of tubular epithelial cells were observed in the DOXR group, significant improvement was observed in the Se and ACA treatment groups. In addition, Se and ACA treatments reduced DOXR-induced 8-OHdG expression ($p< 0.05$). These findings indicated that Se and ACA could be used as critical therapeutic agents to suppress renal dysfunction and oxidative DNA damage that can occur after DOXR-induced kidney injury.

Keywords: 8-OHdG, Doxorubicin, Kidney, N-(p-Amylcinnamoyl) anthranilic acid, Selenium.

ÖZ

Doksorubisin (DOXR) en önemli antitümör ilaçlardan biridir. Bununla birlikte, birçok organda ciddi istenmeyen yan etkileri, DOXR'in klinikteki kullanımını sınırlamaktadır. Bu çalışma, DOXR uygulanan sıçanlarda Selenyum (Se) ve N-(p-Amilsinnamoil) antranilik asit'in (ACA) böbrek dokusu üzerindeki etkisini araştırmayı amaçlamıştır. Çalışmada kullanılacak sıçanlar altı gruba ayrıldı (n=10); Kontrol, DMSO, DOXR, DOXR+Se, DOXR+ACA ve DOXR+Se+ACA. Çalışmanın sonunda, sıçanların intrakardiyak kanı ve ayrıca böbrek dokuları alındı. Sıçanların serum örneklerinde üre ve kreatin seviyelerine bakıldı. Ayrıca böbrek dokusunda histopatolojik inceleme H&E boyama ile ve 8-OHdG ekspresyonu immünohistokimyasal analiz ile belirlendi. Se ve ACA tedavilerinden sonra, serum örneklerinde DOXR ile artan üre ve kreatin seviyeleri azaldı ($p< 0.05$). DOXR uygulanan grupta, glomerüler atrofi, tübüler ve glomerüler dilatasyon, damarlarda tıkanıklık ve tübüler epitel hücrelerinde dejenerasyon gözlenirken, Se ve ACA ile tedavi gruplarda ise önemli derecede düzelme görüldü. Ek olarak Se ve ACA tedavileri, DOXR'in indüklediği 8-OHdG ekspresyonunu azalttı ($p< 0.05$). Bu bulgular, Se ve ACA'nın, DOXR kaynaklı böbrek hasarından sonra meydana gelebilecek böbrek fonksiyon bozukluğunu ve oksidatif DNA hasarını baskılamak için önemli terapötik ajanlar olarak kullanılabileceğini gösterdi.

Anahtar kelimeler: 8-OHdG, Böbrek, Doksorubisin, N-(p-Amilsinnamoil) antranilik asit, Selenyum.

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INTRODUCTION

Doxorubicin (DOXR) is a broad-spectrum antineoplastic agent discovered at the beginning of the 20th century. DOXR treats breast, stomach, thyroid, prostate, testicular, Wilms tumor, Hodgkin lymphoma and many other types of cancer in patients of all age groups (Rivankar, 2014; Wang et al., 2022). It has been stated that the administration of DOXR may cause systemic toxicity, which affects many organs, including the kidney, such as nephrotoxicity or nephritic syndrome (Khames, Gad & Abd El-Raouf, 2017). However, their usefulness in conditions such as cancer, which is extremely serious and still a significant cause of death, allows this disadvantage to be ignored (Chabner et al., 2006). Although different studies examine kidney damage caused by DOXR use (Afsar, Razak, Almajwal, & Al-Disi, 2020; Ibrahim Fouad & Ahmed, 2021), the underlying mechanism is still unknown. Studies on combining preservatives with DOXR are ongoing to reduce DOXR-induced kidney and other tissue injuries.

Selenium (Se) is an essential trace element known as an antioxidant and is involved in various biological processes (Yildizhan, Huyut & Altindag, 2022). Antioxidan defence plays a vital role in various biological processes, including fertility, endocrine function, immune function, carcinogenesis, cardiovascular diseases, and muscle development in both men and women (Ahsan et al., 2014). Today, studies on tissues and organs against the unwanted side effects of DOXR have been performed. Cengiz et al. emphasized that using appropriate doses of Se against DOXR-induced hepatotoxicity reduces liver damage by suppressing pro-inflammatory cytokines (Cengiz et al., 2021). Yang et al. reported that Se supplementation could be a potential therapeutic strategy against DOXR-induced heart damage (Yang, Lu, Yuan, Li & Mao, 2021). It was stated that Se application against the damage of different anticancer drugs to kidney cells (HEK293) has a protective effect by attenuating apoptosis and mitochondrial oxidative stress in cells (Bas & Naziroglu, 2019).

N-(p-Amilcinnamoyl) anthranilic acid (ACA) is a phospholipase-A2 inhibitor and transient receptor potential melastatin-2 (TRPM2) channel blocker (Harteneck, Frenzel, & Kraft, 2007). TRPM2 has an important function in cell viability, and TRPM2 activation is known to increase in response to excessive oxidative stress. Therefore, oxidative stress (OS)-induced activation of the TRPM2 channel may cause damage in healthy cells (Miller, 2019). Cakir et al. determined that against renal ischemia-reperfusion injury, the use of ACA decreased histopathological damage (Cakir et al., 2017).

We did not find any study on the protective effect of Se and ACA against kidney injury caused by DOXR in the literature. Therefore, in this study, we aimed to investigate the effect of Se and ACA on DOXR-induced kidney injury in rats.

MATERIAL AND METHOD

Chemicals

DOXR and ACA and were purchased from TargetMol (Target Molecule Corp., USA). Sodium selenite was purchased from Sigma-Aldrich (Sigma-Aldrich, USA), respectively.

Ethics Statement

This study was conducted at the Van Yuzuncu Yil University Experimental Medicine Research and Application Center after the approval of the Van Yuzuncu Yil University Animal Experiments Local Ethics Committee (decision number: 2022/08-01 and approval date: 01.09.2022).

Experimental Procedure

The rats were divided into six groups (n=10); control, DMSO, DOXR, DOXR+Se, DOXR+ACA and DOXR+Se+ACA. The literature applied the dose and duration for DOXR, Se and ACA (Cakir et al., 2017; Cengiz et al., 2021; Hassan et al., 2020). The experimental protocol of the study is expressed in Figure 1.

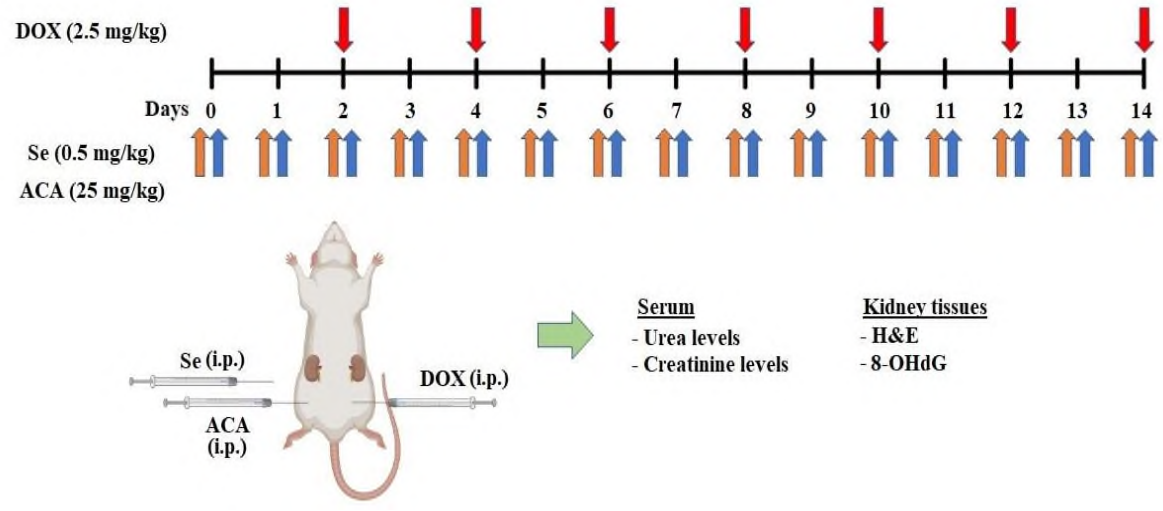

Figure 1. Experimental Protocol of the Study (Created by BioRender).

Measurements of Urea and Creatinine in Serum

The blood sample taken intracardially was centrifuged at 3500 *xg* for 10 min in a dry biochemistry tube to obtain serum samples and stored at -80 °C. Our previous study detailed

the method (Yildizhan et al., 2022). The urea and creatinine in serum were measured spectrophotometrically (Abbott Architect c16000, U.S.A).

Histopathological Analysis

The kidneys were removed under anesthesia and fixed in 10% formaldehyde. After the kidneys undergo, routine histological tissue processes were embedded in paraffin. For histopathological evaluation, sections of 4 μm thickness taken with a microtome were stained with Hematoxylin-Eosin (H&E). The sections were examined under a light microscope with a camera attachment (Olympus BX53, Japan). A mean of 15 fields for each group was evaluated for histopathological evaluation. Histopathological evaluation was scored according to previous studies regarding pathological changes observed in the kidney, such as glomerular atrophy, tubules and glomerular dilatation, vascular congestion and degeneration of epithelial cells (Altınkaynak et al., 2018). 0 = no damage (normal kidney), 1 = less than 25% tissue damage (minimal damage), 2 = 25-50% tissue damage (mild damage), 3 = 50-75% tissue damage (moderate damage), 4 = more than 75% tissue damage (severe damage) (Table 1).

Immunohistochemical Analysis

An immunohistochemical procedure was performed to evaluate the 8-OHdG expression in the kidney tissues. Sections were incubated in an 8-OHdG antibody (Santa Cruz Biotechnology, sc-20067, dilution: 1/50) for one night at +4 ^{o}C. Ten areas were randomly selected for each group animal for the immunohistochemical evaluation. The cells in selected areas were evaluated as negative (0), very low positive (1), low positive (2), moderate positive (3) and high positive (4) cells according to their immune positivity (Table 1).

Statistical Analysis

The SPSS package program was used for biochemical analyzes (version 21). Descriptive statistics for biochemical data were given as mean and standard deviation for the groups. The Shapiro-Wilk test was used to determine whether the data were normally distributed. Since the biochemical data were normally distributed, Duncan test was performed following One Way ANOVA to determine difference between groups. Results with a p-value of 0.05 or less were considered significant.

RESULT

Effect of Se and ACA on Urea Level in DOXR-Induced Kidney Injury

We also measured urea levels in the serum samples. The serum urea levels in the DOXR group were significantly higher than in the other groups ($p< 0.05$). The urea level was lower in the DOXR+Se, DOXR+ACA and DOXR+Se+ACA groups compared with the DOXR group ($p< 0.05$). Urea level was lower in the DOXR+Se+ACA group compared with both DOXR+Se and DOXR+ACA groups ($p< 0.05$). There was no significant difference in urea levels between the control and DMSO groups ($p> 0.05$, Figure 2)

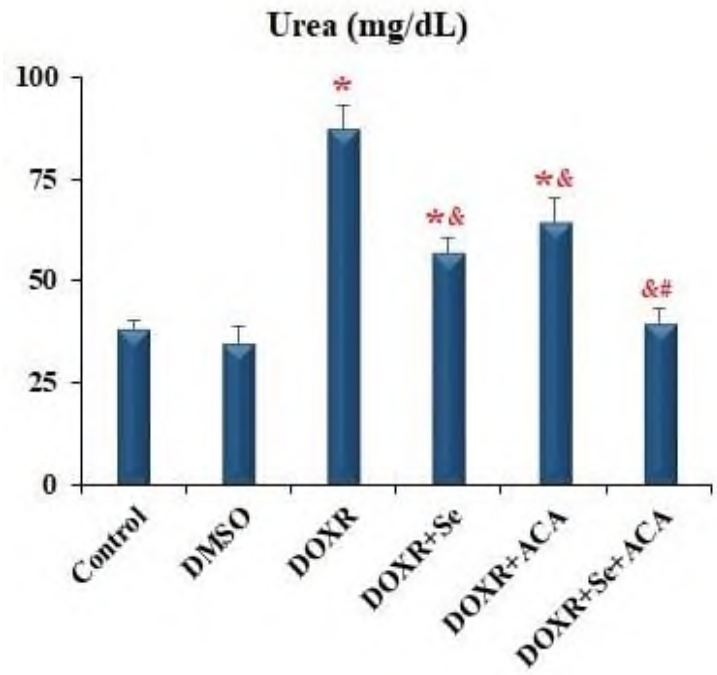

Figure 2. Comparison of Urea Level in Rats' Serum Samples in DOXR-Induced Kidney Injury. (Values are given as mean ± SD., and n=10). (*$p< 0.05$ compared with control and DMSO groups, $^{\&}p<0.05$ compared with DOXR group, $^{\#}p<0.05$ compared with DOXR+Se and DOXR+ACA groups).

Effect of Se and ACA on Creatine Level in DOXR-Induced Kidney Injury

Serum creatine levels were similar in the control and DMSO groups, and the difference between them was insignificant ($p>0.05$). The creatine level of the DOXR group was considerably higher than in the other groups ($p< 0.05$). The creatine level was lower in the DOXR+Se, DOXR+ACA and DOXR+Se+ACA groups compared with the DOXR group ($p< 0.05$). Creatine level was lower in the DOXR+Se+ACA group compared with DOXR+Se and DOXR+ACA groups ($p< 0.05$, Figure 3).

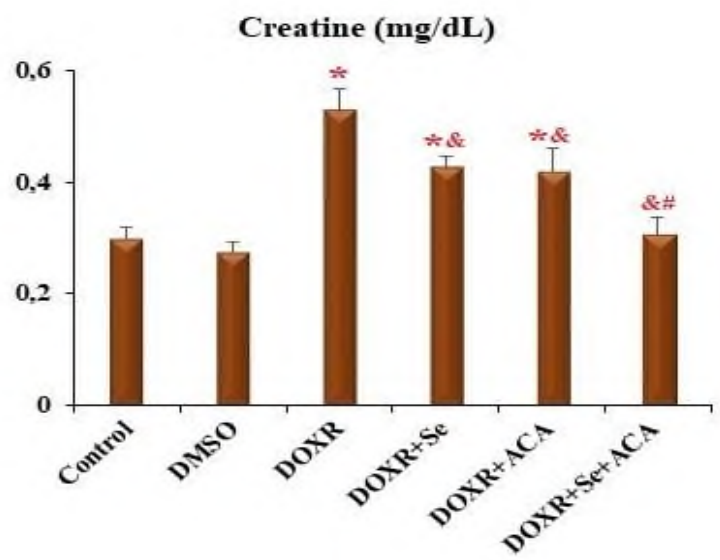

Figure 3. Comparison of Creatine Level in Rats' Serum Samples in DOXR-Induced Kidney Injury. (Values are given as mean ± SD., and n=10). (*p< 0.05 compared with control and DMSO groups, $^{\&}$p<0.05 compared with DOXR group, $^{\#}$p<0.05 compared with DOXR+Se and DOXR+ACA groups).

Histopathological Findings and 8-OHdG Immunoreactivity Results

Microscopic histopathological examinations of kidney tissues were given in Table 1. The kidney tissues of the control (Figure 4A) and DMSO (Figure 4B) groups had stable tubular and glomerular morphological structures. In the group treated with DOXR (Figure 4C), glomerular atrophy, dilatation in tubules and glomerular dilatation, congestion in vessels, and vocalization in tubular epithelial cells were observed. It was observed that these pathological changes improved to varying degrees in DOXR+Se, DOXR+ACA and DOXR+Se+ACA groups (as shown in Table 1, Figure 4D-F).

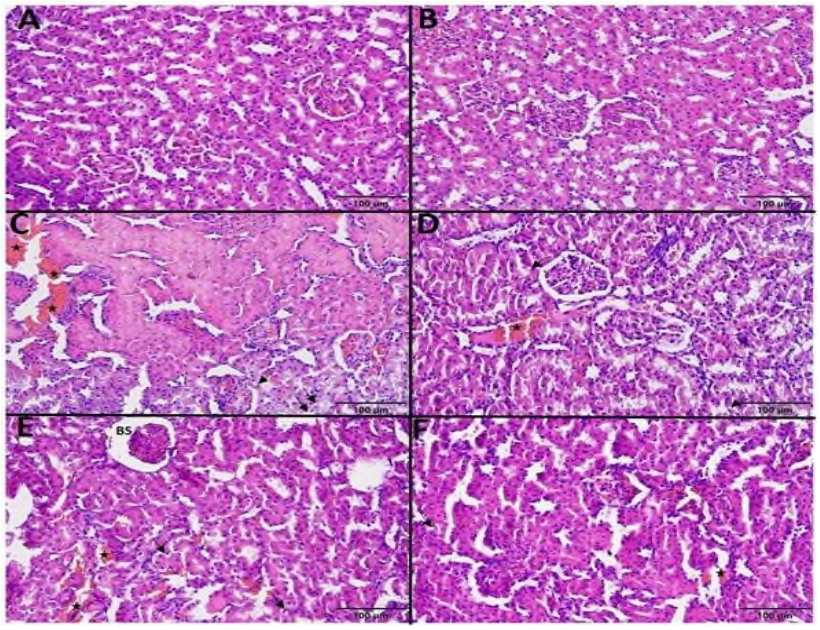

Figure 4. For Histopathological Analyses, Light Microscopic Images of Kidney Tissue Stained with Hematoxylin and Eosin (H&E). Arrow: Degeneration of Tubular Epithelial Cells, Star: Vasocongestion. A: Control, B: DMSO, C: DOXR, D: DOXR+Se, E: DOXR+ACA, F: DOXR+Se+ACA, (Bar: 100 µm).

Table 1. Histopathological and immunohistochemical (IHC) examination scores of kidney tissue belonging to the groups

	Control	DMSO	DOXR	DOXR+Se	DOXR+ACA	DOXR+Se+ACA
Histopathological changes						
Glomerular atrophy	0	0	2	1	2	1
Tubular dilation	0	0	3	1	2	1
Glomerular dilation	0	0	2	1	2	0
Congestion in the veins	0	0	2	1	2	1
Degeneration of tubular epithelial cells	0	0	3	2	2	1
IHC examination						
8-OHdG expression	0	0	3	2	2	1

Table sub-description: The Positivity Degree was Scored According to the Histopathological Findings and the Values in the Table Were Determined as Nominal.

The 8-OHdG expression was negative in the control and DMSO groups (Figures 5A and B). DOXR had a moderate expression of 8-OHdG (Figure 5C), while other treatment groups had low expression of 8-OHdG (Table 1, Figure 5D-F).

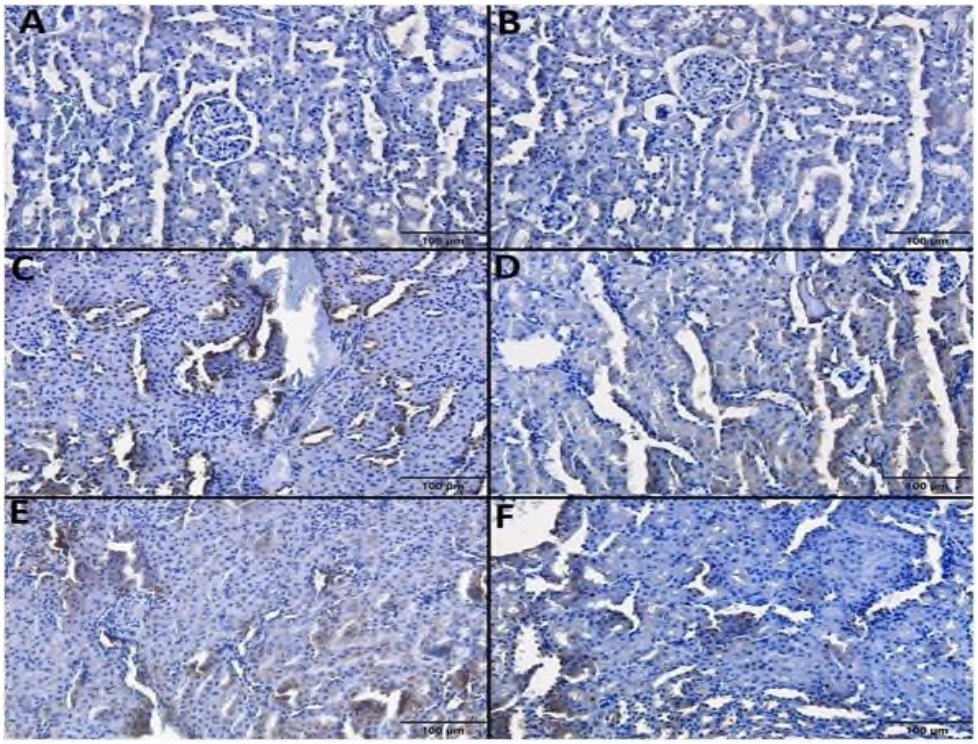

Figure 5. Immunohistochemical Examination Images of 8-OHdG in Kidney Tissue in all the Experimental Groups. A: Control, B: DMSO, C: DOXR, D: DOXR+Se, E: DOXR+ACA, F: DOXR+Se+ACA, (Bar: 100 µm).

DISCUSSION

Although DOXR is one of the most critical antitumor drugs, clinical use of DOXR is limited due to its severe toxicological profile in many organs. Many recent studies showed that DOXR-induced toxicity resulted from lipid peroxidation, oxidative stress, mitochondrial

damage, DNA damage, and apoptosis (Afsar et al., 2020; Ibrahim Fouad & Ahmed, 2021; Qu et al., 2019; Zhao et al., 2018). The most common adverse effects of using DOXR include nephrotoxicity, defined as renal dysfunction with reduced filtration, excretion, and reabsorption associated with a significant risk of morbidity and mortality (AlAsmari et al., 2022). Nephrotoxicity develops in approximately 50% of cancer patients receiving chemotherapy, which unfortunately limits the therapeutic efficacy of DOXR (Fouad & Ahmed, 2021; Fukasawa et al., 2014). Although the fundamental processes that cause DOXR-induced nephrotoxicity are unknown, studies continue to reduce the unwanted side effects and eliminate the restriction on the use of DOXR.

Existing studies have shown that the toxic effects of DOXR administration on the kidney increase renal function parameters, including serum creatinine and urea (Mancilla, Iskra & Aune, 2019). Hekmat et al. found a significant increase in urea and creatinine levels in DOXR-induced rats. In contrast, serum urea and creatine levels decreased parallel with the decrease in kidney damage in the groups treated (Hekmat, Navabi, Alipanah & Javanmardi, 2021). A different study noted that linalool may be used therapeutically and protectively in DOXR-induced kidney injury and regulated the high serum urea and creatine levels caused by DOXR (Altinoz, Oner, Elbe, Uremis & Uremis, 2021). Fouad et al. found that administering a single dose of DOXR (20 mg/kg, i.p.) caused nephrotoxicity and renal fibrosis. In addition, the same study determined that berberine application with DOXR decreased nephrotoxicity. Furthermore, the DOXR-induced serum urea and creatine levels were significantly reduced in the berberine+DOXR group compared with the DOXR group (Fouad & Ahmed, 2021). In the study of Ali et al., DOXR was combined with compounds exhibiting antioxidant properties to minimise DOXR side effects. They reported that the antioxidant level was low and also worsened kidney function tests after DOXR administration. They also demonstrated that diosmin, known for its antioxidant properties, reduced DOXR-induced nephrotoxicity (Ali et al., 2021). In our research, in parallel with the literature data, we observed an increase in urea and creatine levels in rat sera after DOXR administration. Also, we determined that the Se or ACA treatment against this harmful effect of DOXR decreased serum urea and creatine levels. Furthermore, the combination of Se and ACA reduced the urea and creatine levels to the level of the control group (Figures 2 and 3).

In tissue toxicity studies, it is also important to evaluate whether the tissue is in a normal structure (Uçar, Huyut, Altındağ, Keleş & Yıldızhan, 2022; Ugur et al., 2015). Altınkaynak et al. reported that unicellular necrosis, tubular atrophy, tubular necrosis, and glomerular necrosis were seen in the kidneys of DOXR-applied rats (Altınkaynak et al., 2018). In another study, it

was reported that glomerular atrophy, congestion, inflammatory cell infiltration, and proximal tubule damage were observed in the histopathological examination in the kidneys of DOXR-applied rats (Hosseinzadeh et al., 2020). Cardoso et al. reported that histopathological examination of kidney tissue of DOXR-applied rats that they demonstrated a significantly altered kidney structure with signals of cellular degeneration, necrosis, and inflammation, a general collagen deposition, and an increase in Bowman's capsule thickness (Cardoso, Coriolano & Duarte, 2018). In our study, the kidney of the control and DMSO groups had a normal histological structure. In contrast, severe glomerular atrophy, tubular dilation, glomerular dilation, congestion in the veins, and degeneration of tubular epithelial cells were observed in the kidney tissue of the rats in the DOXR group. Compared with the DOXR group, these histological changes were regenerated in the DOXR+Se and DOXR+ACA groups, while the DOXR+Se+ACA group had minor histological changes compared with the DOXR-treated groups (Table 1 and Figure 4).

8-OHdG is an important fundamental biomarker to measure endogenous oxidative DNA damage (Valavanidis, Vlachogianni, & Fiotakis, 2009). Khan et al. examined the multiple side effects of DOXR in mice. They showed that the DOXR group significantly increased 8-OHdG levels compared with the treatment groups. In addition, they noted that green synthesized selenium nanoparticles used for therapeutic purposes showed a protective effect against DOXR-induced damage (Khan et al., 2022). In our research, in parallel with the literature data, we saw that 8-OHdG expression was negative in the control and DMSO groups (Figures 5A and B), while the DOXR group had moderate 8-OHdG expression (Figure 5C). Furthermore, we observed that the other treatment groups had lower 8-OHdG expression than the DOXR group (Table 1, Figure 5D-F).

CONCLUSION

This study showed that DOXR administration increased serum samples' total urea and creatine levels and increased oxidative damage in kidney tissue. However, it also indicates that using Se and ACA in conjunction with DOXR administration restores and reduces kidney damage and oxidative damage. These results showed that the application of Se and ACA after DOXR administration could suppress DOXR-induced kidney damage and oxidative damage. However, there is a need for studies investigating the signalling pathways at the molecular level about how DOXR increases kidney function tests and oxidative DNA damage and how ACA and Se inhibited these parameters and kidney damage.

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REFERENCES

Afsar, T., Razak, S., Almajwal, A. & Al-Disi, D. (2020). Doxorubicin-induced alterations in kidney functioning, oxidative stress, DNA damage, and renal tissue morphology; Improvement by Acacia hydaspica tannin-rich ethyl acetate fraction. *Saudi Journal of Biological Sciences,* 27(9), 2251-2260.

Ahsan, U., Kamran, Z., Raza, I., Ahmad, S., Babar, W., Riaz, M. & Iqbal, Z. (2014). Role of selenium in male reproduction-A review. *Animal Reproduction science,* 146(1-2), 55-62.

AlAsmari, A. F., Ali, N., Alharbi, M., Alqahtani, F., Alasmari, F., Almoqbel, D., …Al-Harbi, N. O. (2022). Geraniol Ameliorates Doxorubicin-Mediated Kidney Injury through Alteration of Antioxidant Status, Inflammation, and Apoptosis: Potential Roles of NF-kappaB and Nrf2/Ho-1. *Nutrients,* 14(8), 1620.

Ali, N., AlAsmari, A. F., Imam, F., Ahmed, M. Z., Alqahtani, F., Alharbi, M., AlSwayyed, M., AlAsmari, F., Alasmari, M., Alshammari, A., Fantoukh, O. I. & Alanazi, M. M. (2021). Protective effect of diosmin against doxorubicin-induced nephrotoxicity. *Saudi J Biol Sci,* 28(8), 4375-4383.

Altınkaynak, Y., Kural, B., Akcan, B. A., Bodur, A., Özer, S., Yuluğ, E., …Örem, A. (2018). Protective effects of L-theanine against doxorubicin-induced nephrotoxicity in rats. *Biomedicine & Pharmacotherapy,* 108, 1524-1534.

Altinoz, E., Oner, Z., Elbe, H., Uremis, N. & Uremis, M. (2021). Linalool exhibits therapeutic and protective effects in a rat model of doxorubicin-induced kidney injury by modulating oxidative stress. *Drug Chem Toxicol,* 45 (5), 2024-2030.

Bas, E. & Naziroglu, M. (2019). Selenium attenuates docetaxel-induced apoptosis and mitochondrial oxidative stress in kidney cells. *Anti-Cancer Drugs,* 30(4), 339-346.

Cakir, M., Duzova, H., Tekin, S., Taslidere, E., Kaya, G. B., Cigremis, Y., …Yologlu, S. (2017). ACA, an inhibitor phospholipases A2 and transient receptor potential melastatin-2 channels, attenuates okadaic acid induced neurodegeneration in rats. *Life Sci*, 176, 10-20.

Cardoso, D. F., Coriolano, H.-J. A. & Duarte, J. A. (2018). Regular voluntary running has favorable histological effects on doxorubicin-induced kidney toxicity in Wistar rats. *Cell and Tissue Research,* 374(1), 177-187.

Cengiz, O., Baran, M., Balcioglu, E., Suna, P. A., Bilgici, P., Goktepe, O., Onder, G. O., Goc, R. & Yay, A. (2021). Use of selenium to ameliorate doxorubicin induced hepatotoxicity by targeting pro-inflammatory cytokines. *Biotech Histochem,* 96(1), 67-75.

Chabner, B. A., Amrein, P. C., Druker, B. J., Michaelson, M. D., Mitsiades, C. S., Goss, P. E., …Supko, J. G. (2006). Antineoplastic agents. *The Pharmacological Basis of Therapeutics 9/e,* 1315-1465.

Fouad, G. I. & Ahmed, K. A. (2021). The protective impact of berberine against doxorubicin-induced nephrotoxicity in rats. *Tissue and Cell,* 73, 101612.

Fukasawa, H., Furuya, R., Yasuda, H., Yamamoto, T., Hishida, A. & Kitagawa, M. (2014). Anti-cancer agent-induced nephrotoxicity. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents),* 14(7), 921-927.

Harteneck, C., Frenzel, H. & Kraft, R. (2007). N-(p-amylcinnamoyl) anthranilic acid (ACA): a phospholipase A2 inhibitor and TRP channel blocker. *Cardiovascular Drug Reviews,* 25(1), 61-75.

Hassan, M. Q., Akhtar, M. S., Afzal, O., Hussain, I., Akhtar, M., Haque, S. E. & Najmi, A. K. (2020). Edaravone and benidipine protect myocardial damage by regulating mitochondrial stress, apoptosis signalling and cardiac biomarkers against doxorubicin-induced cardiotoxicity. *Clinical and Experimental Hypertension*, 42(5), 381-392.

Hekmat, A. S., Navabi, Z., Alipanah, H. & Javanmardi, K. (2021). Alamandine significantly reduces doxorubicin-induced cardiotoxicity in rats. *Hum Exp Toxicol*, 40(10), 1781-1795.

Hosseinzadeh, A., Goudarzi, M., Fatemi, I., Khodayar, M. J., Mehrzadi, S., Khalili, H. R. & Karimi, M. A. (2020). Gemfibrozil attenuates doxorubicin induced toxicity in renal tissues of male rats by reducing the oxidative insult and inflammation. *Biotechnic & Histochemistry*, 95(7), 532-539.

Ibrahim Fouad, G. & Ahmed, K. A. (2021). The protective impact of berberine against doxorubicin-induced nephrotoxicity in rats. *Tissue Cell*, 73, 101612.

Khames, A., Gad, A. M. & Abd El-Raouf, O. M. (2017). Ameliorative effects of sildenafil and/or febuxostat on doxorubicin-induced nephrotoxicity in rats. *European Journal of Pharmacology*, 805, 118-124.

Khan, M. A., Singh, D., Arif, A., Sodhi, K. K., Singh, D. K., Islam, S. N., …Siddique, H. R. (2022). Protective effect of green synthesized Selenium Nanoparticles against Doxorubicin induced multiple adverse effects in Swiss albino mice. *Life Sciences*, 305, 120792.

Mancilla, T. R., Iskra, B. & Aune, G. J. (2019). Doxorubicin-induced cardiomyopathy in children. *Comprehensive Physiology*, 9(3), 905.

Miller, B. A. (2019). TRPM2 in Cancer. *Cell Calcium*, 80, 8-17.

Qu, X., Zhai, J., Hu, T., Gao, H., Tao, L., Zhang, Y., Song, Y. & Zhang, S. (2019). Dioscorea bulbifera L. delays the excretion of doxorubicin and aggravates doxorubicin-induced cardiotoxicity and nephrotoxicity by inhibiting the expression of P-glycoprotein in mice liver and kidney. *Xenobiotica*, 49(9), 1116-1125.

Rivankar, S. (2014). An overview of doxorubicin formulations in cancer therapy. *J Cancer Res Ther*, 10(4), 853-858.

Uçar, B., Huyut, Z., Altındağ, F., Keleş, Ö. F. & Yıldızhan, K. (2022). Relationship with nephrotoxicity of Abemaciclib in rats: Protective effect of Curcumin. *IJBB*, 59(10), 963-976.

Ugur, S., Ulu, R., Dogukan, A., Gurel, A., Yigit, I. P., Gozel, N., Aygen, B. & Ilhan, N. (2015). The renoprotective effect of curcumin in cisplatin-induced nephrotoxicity. *Renal Failure*, 37(2), 332-336.

Valavanidis, A., Vlachogianni, T. & Fiotakis, C. (2009). 8-hydroxy-2′-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. *Journal of Environmental Science and Health Part C*, 27(2), 120-139.

Wang, Q., Wang, C., Li, S., Xiong, Y., Wang, H., Li, Z., Wan, J., Yang, X. & Li, Z. (2022). Influence of linkers within stimuli-responsive prodrugs on cancer therapy: A case of five doxorubicin dimer-based nanoparticles. *Chemistry of Materials*, 34(5), 2085-2097.

Yang, H. B., Lu, Z. Y., Yuan, W., Li, W. D. & Mao, S. (2021). Selenium Attenuates Doxorubicin-Induced Cardiotoxicity Through Nrf2-NLRP3 Pathway. *Biol Trace Elem Res*. 200, 2848-2856.

Yildizhan, K., Huyut, Z. & Altindag, F. (2022). Involvement of TRPM2 channel on doxorubicin-induced experimental cardiotoxicity model: Protective role of selenium. *Biol Trace Elem Res*., in 04 August 2022 retrieved from https://link.springer.com/article/10.1007/s12011-022-03377-2#article-info.

Zhao, L., Qi, Y., Xu, L., Tao, X., Han, X., Yin, L. & Peng, J. (2018). MicroRNA-140-5p aggravates doxorubicin-induced cardiotoxicity by promoting myocardial oxidative stress via targeting Nrf2 and Sirt2. *Redox Biol*, 15, 284-296.